

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Roberts *et al.*

Serial No.: 10/601,841

Docket No.: 032026-0731

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Examiner: M. Perreira

For: SYNTHESIS OF ^{17}F LABELED FLUOROALKANES

DECLARATION OF ROBERT J. NICKLES

I, Robert J. Nickles (Jerry Nickles), do hereby declare and state as follows:

I am a professor of medical physics, radiology and physics at the University of Wisconsin-Madison (UW-Madison). I have held my current position since 1981.

I received a Bachelors of Science in Math and Physics from UW-Madison in 1962, a Masters in Science in Nuclear Physics from the University of Sao Paulo in 1967, and a Ph.D. in Nuclear Physics from UW-Madison in 1968.

A copy of my professional biography is attached.

I am a coinventor of the subject matter claimed in the above-referenced patent application.

I have reviewed the disclosures of Ruth et al., Synthesis of C-11 and F-18 Labelled Compounds for Biomedical Applications: Current Status and Challenges for the Future in the Journal of Radioanalytical and Nuclear Chemistry, Vol. 203, No. 2, pages 457-469 (1996) (hereinafter "Ruth") and Pike et al., Novel Use of an Isotope Separator to Determine the Position of Fluorine-18 in Labelled 1,1,1,2-Tetrafluoroethanes in Organic Mass Spectrometry, Vol. 29, pages 499-504 (1994) (hereinafter "Pike").

On pages 462-465 Ruth describes electrophilic and nucleophilic fluorination reactions used in ^{18}F labeling. On page 500 Pike describes nucleophilic fluorination reactions used in ^{18}F labeling 1,1,1,2-tetrafluoroethane. Neither the electrophilic reactions described in Ruth nor the nucleophilic reactions described in Ruth and Pike could be used to incorporate ^{17}F into organic molecules to produce a radiopharmaceutical for imaging use.

Conventional electrophilic reactions for ^{18}F labeling organic molecules, including the electrophilic reactions described in Ruth, could not be used to produce an ^{17}F labeled radiopharmaceutical for imaging use because they involve reacting (bubbling) the gas phase $^{18}\text{F}_2$, diluted in an inert gas with a precursor, generally a mercurated or stannylated compound, dissolved in a suitable solvent such as chlorofluoromethane. After the electrophilic demetallation, the subsequent steps involve flash chromatography to remove the alkyl-stannyl

group, evaporation of the organic solvent, hydrolysis, and HPLC clean-up prior to the final radiopharmaceutical steps. These bubbling, alkyl-stannyl removal, hydrolysis, and HPLC steps generally require times of the order of tens of minutes, typically 40 minutes to make ^{18}F -fluoro-DOPA. This time scale is completely impractical with ^{17}F , with a 64 second half life.

Conventional nucleophilic reactions for ^{18}F labeling organic molecules, including the nucleophilic reactions described in Ruth and Pike, could not be used to produce an ^{17}F labeled radiopharmaceutical for imaging use because of the same unfavorable time scale. Here, the situation is even worse, with standard nucleophilic labeling conditions starting with aqueous ^{18}F -fluoride, separated from the ^{18}O -enriched target water by a trap and release column. The ^{18}F -activity is then supported by a phase transfer catalyst such as Kryptofix 2.2.2 / K^+ in acetonitrile / water, which is then azeotropically distilled to finally reveal the supported ^{18}F activity in an organic solvent (e.g. acetonitrile) with rigorous exclusion of water. The nucleophilic attack can then proceed with the chosen precursor material, followed by solid phase (Sep-pak) extraction, hydrolysis and deprotection, for final clean-up. These steps, particularly the removal of all traces of water from the Kryptofix/ K^+F^- require tens of minutes under gentle conditions, totally impractical for the 64-second half life of ^{17}F . For these reasons, the fast, flow-through ("jet") chemistry that has been developed for our production of the ^{17}F -fluoroalkanes, as described in the above-referenced patent application, is unique and is not discussed in the cited literature.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Nov 13, 2006

Signature:

Robert J. Nickles
Robert J. Nickles

Jerry Nickles, PhD.

Professor of Medical Physics, Radiology, and Physics.

B.S. in Math/Physics from the University of Wisconsin-Madison, 1962.

M.S. in Nuclear Physics from the University of Sao Paulo, 1967.

Ph.D. in Nuclear Physics from the University of Wisconsin-Madison, 1968.

His office phone number is (608) 263-1026.

E-mail him at nickles@petrus.medphysics.wisc.edu.

Professional Experience

1968-1969 Postdoctoral Fellow, Nuclear Physics, University of Wisconsin, Madison

1969-1972 James A. Picker Fellow, Niels B?Institute, Copenhagen, Denmark

1972-1976 Assistant Professor of Radiology, University of Wisconsin, Madison

1976-1981 Research Career Development Award

1976 Visiting Professor, University of Groningen, Netherlands

1976-1981 Associate Professor of Radiology, University of Wisconsin, Madison

1977 Visiting Professor, University of Sao Paulo, Brazil

1980 Exchange Visiting Professor, University of Helsinki, Finland

1981-present Professor of Medical Physics and Radiology, University of Wisconsin-Madison

1991 Exchange Visiting Professor, Paul Scherrer Institut, Villigen, Switzerland

Research Interests

My research interests have focused on the production and application of positron emitters in radionuclide imaging. While historically PET is linked to the four major radioisotopes ^{11}C , ^{13}N , ^{15}O and ^{18}F , the work at Wisconsin have broadened this list to dozens of new candidates, opening up unique possibilities for labeling agents with

- very short-lived tracers, such as ^{10}C , ^{14}O and ^{17}F for tracking blood flow
- transition metals (Mn, Cu,...) well-suited for chelation chemistry
- positron-emitting ^{94m}Tc to bridge the gap between PET and SPECT
- and scaled up production to satisfy diverse needs, such as studies with radioactive ion beams.

The application of these agents centers on the integrated resources of the UW Medical Physics Dept, including the first prototype CTI 11 MeV proton cyclotron, serving a fully equipped radiochemistry lab, feeding a CTI 933/04 ECAT scanner dedicated to basic imaging research. Instrument development underpinning these facilities has been an integral part of the dissertation projects of 15 PhD candidates graduating from the cyclotron group, and as many excellent MS students.

Recent publications

Nickles RJ, Christian BT, Mulnix TL, Stone CK: Quantitating Technetium Pharmacokinetics with PET, SPECT, and Beta Spectroscopy. J. Lab. Comp. Radiopharm. XXXV, 22-25 (1994).

Nickles RJ, DeJesus OT: Truly Frugal PET: Is It Possible? in Chemists View of Imaging Centers, pp133-139, A.M.Emran (ed), Plenum Press, New York, NY, (1994).

Satter MR, Martin CC, Oakes TR, Christian BT, Nickles RJ: Synthesis of the Fluorine-18 Labeled Inhalation Anesthetics. Int. J. Appl. Rad. Isotopes 45(11), 1093-1100 (1994).

- Hochachaka PW, Clark CM, Brown WD, Stanely C, Stone CK, Nickles RJ, Zhu G, Allen PS, Holden JE: The Brain at High Altitude: Hypometabolism as a Defense Against Chronic Hypoxia? *J. Cereb. Bl. Flow Metab.* 14: 671--679 (1994)
- Roberts AD, Daniel LC, Nickles RJ: A High Power Target for the Production of [F-18]-fluoride. *Nucl. Inst. Methods B* 99, 797-799 (1995).
- Roberts AD, Oakes TR, Nickles RJ: Development of an Improved Target for [F-18]F₂ Production. *Int. J. Appl. Rad. Isotopes* 46, 87-91 (1995).
- Christian BT, Nickles RJ, Mulnix TL, Stone CK: The Production of ^{94m}Tc for PET Imaging. *Int. J. Appl. Rad. Isotopes* 46 69-73 (1995).
- Roberts AD, Nickles RJ, Paul M, Rehm KE, Jiang CL, Blumenthal DJ, Gehring J, Henderson D, Nolen J, Pardo RC, Schiffer JP, Segel RE: Production of a Radioactive F-18 Ion Beam for Nuclear Reaction Studies. *Nucl. Instr. Methods B* 103(4), 523-528 (1995).
- Holden JE, Stone CK, Clark CM, Brown WD, Nickles RJ, and Hochachaka PW, "Enhanced Cardiac Metabolism of Plasma Glucose in High-Altitude Natives: Adaptation against Chronic Hypoxia," *J Appl Physiol* 79, 222-238 (1995).
- Martin CC, Christian BT, Satter MR, Nickerson LDH, and Nickles RJ, "Quantitative PET with Positron Emitters That Emit Prompt Gamma Rays," *IEEE Trans. Nucl. Sci.* 14(4), 681-687 (1995).
- Nickles RJ, Mulnix TL, Christian BT, Stone CK, and Nunn AD, "A Triple Isotope Technique to Study the Radiopharmacology of Technetium Agents," *J Lab Comp Radiopharm* 37, 726-9 (1995).
- DeJesus OT, Murali D, and Nickles RJ, "Synthesis of Brominated and Fluorinated Ortho-Tyrosine Analogs as Potential DOPA Decarboxylase Tracers," *J. Lab. Comp. Radiopharm* 37, 147-149 (1995).
- Rehm KE, Paul M, Roberts AD, Blumenthal, Gehring J, Henderson D, Jiang CL, Nickles J, Nolen J, Pardo RC, Schiffer JP, and Segel RE, "Study of the ¹⁸F(p,a)¹⁵O Reaction at Astrophysical Energies Using a ¹⁸F Beam," *Phys Rev C* 52, R460-3 (1995).
- Taylor MD, Mulnix TL, Roberts AD, Andreaco MS, Williams CW, and Nickles RJ, "PETite: a Tomographic Test Bench," *IEEE Trans. Nucl. Sci. Suppl.* 1, 65-68 (1995).
- Taylor M, Roberts AD, and Nickles RJ, "Improving Yield of ²FDG Using a Microwave Cavity," *Nucl. Med. Biol.* 23, 605 (1996).
- Rehm KE, Paul M, Roberts A, Blumenthal D, Gehring J, Henderson D, Jiang CL, Nickles RJ, Nolen J, Pardo RC, Schiffer JP, and Segel RE, "The Astrophysical Reaction Rate for the ¹⁸F(p,α) ¹⁵O Reaction", *Phys. Rev. C* 53, 438 (1996).
- Tewson TJ, Yang D, Wong G, Macy D, DeJesus OJ, Nickles RJ, Perlman SB, Taylor MD, Frank P: The Synthesis of F-18 Lomefloxacin and Its Preliminary Use in Human Studies. *Nucl. Med. Biol.* 23, 767-772 (1996).
- Rehm KE, Jiang CL, Paul M, Blumenthal D, Gehring J, Henderson D, Nickles J, Nolen J, Pardo RC,

- Roberts AD, Schiffer JP, Segel RE: The Use of a Gas-filled Magnet in Experiments with Radioactive Ion Beams. *Nucl. Instr. Methods A* 370, 438 (1996).
- Hochachka P.W., Clark C.M., Monge C., Stanley C., Brown W.D., Stone C.K., Nickles R.J., and Holden J.E., "Sheep Brain Glucose Metabolism and Defense Adaptations Against Chronic Hypoxia," *J. Applied Physiol.* 81, 1355-1361 (1996).
- Nickles-RJ, Kulago-AA, Schueller-MJ, and Stone-CK, "A Rather Intense Source of Fast Neutrons for Small Scale Activation." *J Appl Radiat Isotopes* 48, 55-59 (1997).
- DeJesus OT, Lefebvre PA, Chyan M-K, Nickles RJ. Synthesis of F-18 -alpha-Fluoro Methyl Phenylalanine: A Potential Tyrosine Hydroxylase Tracer. *J Lab Radiopharm.* 40, (1997) 674-676.
- DeJesus OT, Endres CJ, Shelton SE, Nickles RJ, and Holden JE, "Evaluation of Fluorinated m-Tyrosine Analogs as PET Imaging Agents of Dopamine Nerve Terminals: Comparison with 6-FluoroDOPA." *J Nucl Med* 38, 630-635 (1997).
- Rehm K.E., Jiang C.L., Paul M., Blumenthal D., Daniel L.C., Davids C.N., DeCrock P., Fischer S.M., Henderson D., Lister C., Nickles R.J., Nolen J., Pardo R.C., Schiffer J.P., Seweryniak D., and Segel R.E., "Exploring the 18F Gateway to the Formation of Heavy Elements in Hot Stars," *Phys. Rev. Letters* 55, R566-569 (1997).
- Renstrom B., Rommelfanger S., Stone C.K., DeGrado T.R., Carlson K.J., Scarbrough E., Nickles R.J., , Liedtke A.J., and Holden J.E., "Comparison of Fatty Acid Tracers FTHA and BMIPP During Myocardial Ischemia and Hypoxia," *J. Nucl. Med* 39, 1684-1689 (1998).
- Stone C.K., Pooley R.A., DeGrado T.R., Nellis S.H., Nickles R.J., Renstrom B., Liedtke A.J., and Holden J.E., Retention of Fatty Acid Tracers FTHA and BMIPP During Myocardial Ischemia and Hypoxia," *J. Nucl. Med* 39, 1690-1696 (1998).
- Brown W.D., Oakes T.R., DeJesus O.T., Taylor M.D., Roberts A.D., Nickles R.J., and Holden J.E., "Dosimetry for F-18 Fluoro-L-DOPA with Carbidopa Pretreatment Using the MIRD Pamphlet 14 Bladder Model," *J. Nucl. Med.* 39, 1884-1897, 1998.
- DeJesus O.T., Lefebvre P.A., Chyan M-K., and Nickles R.J., "Synthesis of F-18-a-Fluoro Methyl Phenylalanine: A Potential Tyrosine Hydroxylase Tracer," *J. Lab. Comp. Radiopharm* 40,(1997) 674-676.
- Taylor M.D. and Nickles R.J., "F-18 Halothane Photolabeling of Isolated Proteins," *J. Lab. Comp. Radiopharm.* 40, (1997) 11-15.
- Nickles R.J., Taylor M.D., Mulnix T.L., Roberts A.D., Schueller M.J., Kulago A.A., DeJesus O.T., Brown W.D., Whitesell L.F., Carlson K.C., and Stone C.K., "Achieving Balanced Resolution in Radiopharmacological Studies: Tc-Labeled BMS 194,796," *J. Lab. Comp. Radiopharm.* 40, (1997) 139-142.
- Rehm K.E., Jiang C.L., Paul M., Blumenthal D., Daniel L.C., Davids C.N., DeCrock P., Fischer S.M., Henderson D., Lister C., Nickles R.J., Pardo R.C., Schiffer J.P., Seweryniak D., and Segel R.E., "Study of Radiative Capture with Radioactive Ion Beams," *Nucl. Instr. Meth. A* 418,(1998) 355-364.
- Wallhaus TR, Lacy J, Whang J, Green MA, Nickles RJ, Stone CK. Human Biodistribution and

Dosimetry of the Perfusion Agent Copper-62 PTSM. J Nucl Med 39, (1998) 1958-1964.

Roberts AD, Davidson RJ, Nickles RJ. Production of F-17, O-15 and Other Radioisotopes for PET Using a 3 MV Electrostatic Tandem Accelerator. AIP Conf Proc 415 (1999).

Hoachachka PW, Clark CM, Matheson GO, Brown WD, Stone CK, Nickles RJ, Holden JE. Effects on Regional Brain Metabolism of Prolonged :High Altitude Hypoxia: A Study of Six US Marines. Am J Physiol 46, (1999) R314-319.

DeJesus OT, Brown WD, Shelton SE, Roberts AD, Nickles RJ, Holden JE. Studies in Rhesus Monkeys Using 6-Fluoro Meta Tyrosine: an Aromatic L-Amino Acid Decarboxylase Tracer. J Lab Radiocompounds 42, (1999) S392-394.

Nickles RJ, Jensen M, Paans AMJ, Holm S, Schueller MJ, Vaalburg W. Doppler Broadening Revealing the Chemical Environment Following Oxygen-14 Decay. (Radiochimica Acta in press).

Brown WD, Taylor MD, Roberts AD, Oakes TR, Schueller MJ, Holden JE, Malischke LM, DeJesus OT, Nickles RJ. FluoroDOPA PET Shows the Non-dopaminergic as well as Dopaminergic Desitinations of Levodopa. Neuroliogy 53,(1999) 1212-18.

Brown WD, deJesus OT, Pyzalski RW, Malischke LM, Roberts AD, Shelton SE, Uno H, Houser WD, Nickles RJ, Holden JE. Localization and Trapping of ^{18}F -m-tyrosine, an AAAD Tracer for PET. Synapse 34, (1999) 111-123.

Nickles RJ. A Positron Floodlight for PET Attenuation Measurements.(submitted to IEEE Trans Nucl Sci.)

Alves F, Jensen M, Jensen HJ, Nickles RJ, Holm S. Determination of the B(p,n)C-10 Excitation Function with Implications for the Production of C-10 Carbon Dioxide as a PET Tracer. J Appl Rad Isotopes 52 (2000), 899-903.